ORIGINAL ARTICLE

# Serum antioxidant vitamins and the risk of oral cancer in patients seen at a tertiary institution in Nigeria

AO Lawal, B Kolude, BF Adeyemi, JO Lawoyin, EE Akang[1]

Departments of Oral Pathology and [1]Pathology, College of Medicine, University of Ibadan, Nigeria

## Abstract

**Objectives:** Tobacco and alcohol are major risk factors of oral cancer, but nutritional deficiency may also contribute to development of oral cancer. This study compared serum antioxidant vitamin levels in oral cancer patients and controls in order to validate the role of vitamin deficiencies in the etiology of oral cancer.

**Materials and Methods:** Serum vitamin A, C, and E levels of 33 oral cancer patients and 30 controls at University College Hospital, Ibadan, Nigeria, were determined using standard methods. The data obtained were analyzed using the Student t-test, odds ratio, and logistic regression.

**Results:** Mean vitamin A, C, and E levels were significantly lower in oral cancer patients ($P$=0.022, $P$=0.000, and $P$=0.013 respectively). Risk of oral cancer was 10.89, 11.35, and 5.6 times more in patients with low serum vitamins A, C, and E, respectively. However, on logistic regression analysis, only low serum vitamin E independently predicted occurrence of oral cancer.

**Conclusions:** The lower serum vitamin A, C, and E levels in oral cancer patients could be either a cause or an effect of the oral cancer. Further studies using a larger sample size and cohort studies with long-term follow-up of subjects are desirable.

**Key words:** Antioxidant vitamins, Nigeria, oral cancer risk

**Date of Acceptance:** 05-Jun-2011

## Introduction

Oral cancer accounts for between 2% and 4% of all malignant tumors in most regions of the world, and constitutes a great challenge for the afflicted person and for healthcare professionals.[1,2] The trend for survival of patients with oral cancer has remained rather disappointing over the past several decades, with the overall 5 years survival rate being approximately 50%.[3,4]

Tobacco and alcohol are the most important etiological factors in the development of oral cancer.[2,5,6] However, some authors have reported oral cancer in people who do not use tobacco or consume any appreciable amounts of alcohol.[7,8] This suggests that other important factors might contribute to the etiology of oral cancer.

**Address for correspondence:**
Dr. AO Lawal,
Department of Oral Pathology, College of Medicine,
University of Ibadan, Nigeria.
E-mail: toytoy219@yahoo

Experimental and clinical studies have shown that antioxidant vitamins may inhibit cancer formation and progression. Suda *et al.*[9] showed that topically applied beta-carotene inhibited experimental oral carcinogenesis in hamster pouch. Odukoya *et al.*[10] also showed that topically applied vitamin E inhibited carcinogenesis in hamster buccal pouch. Other studies have shown that diets high in fruits and vegetables, vitamin A, and vitamin C have a protective effect against oral cancer.[11,12]

Clinical studies in the United States[13] and Japan[14] also found lower serum antioxidant vitamins in oral cancer patients compared with normal population.

| Access this article online | |
|---|---|
| Quick Response Code: | **Website:** www.njcponline.com |
| | **DOI:** 10.4103/1119-3077.94093 |
| | **PMID:** 22437085 |

Oji *et al.*[15] in Enugu reported that most of the oral cancer patients presenting at the University of Nigeria Teaching Hospital over a 6-year period gave a negative history of alcohol and tobacco exposure. They suggested that poverty, malnutrition, lack of education, poor oral hygiene, and chronic malaria might play an important role in etiology and severity of oral cancer. Their finding is in agreement with that of Lawoyin *et al.*[16] who in their study in south-western Nigeria, found low prevalence of recognized risk factors for oral cancer such as tobacco and alcohol consumption in their study population and suggested thatother predisposing factors such as nutrition, genetic predisposition, and chronic illness may play an important role in the etiology of oral cancer in their study population.

The aim of this study was to analyze the serum antioxidant vitamins in oral cancer patients and compare them with those of controls in order to validate the probable role of vitamin deficiencies in the etiology of oral cancer in a Nigerian population.

## Materials and Methods

Thirty healthy volunteers and —33 histologically diagnosed oral cancer patients participated in this study. All cases were patients from the outpatients department of the Dental Clinic of the University College Hospital, Ibadan in south-western Nigeria. Ethical clearance was obtained from the joint ethical committee of University of Ibadan and the University College Hospital. All patients were duly informed of the aim of the procedure and consented to participate in the study. Healthy volunteers under age 40 years and all those with known systemic diseases were excluded from the study. Patients who had commenced any form of radiotherapeutic, chemotherapeutic or surgical interventions were excluded from the study.

Ten milliliters of intravenous blood was taken from all participants after an overnight fast. The blood was centrifuged at 3000 rpm for 5 minutes and separated serum was aspirated into tubes and analyzed for vitamins A, C, and E with a DM520 spectrophotometer (Beckman, USA). The spectrophotometer measures the concentrations of the different vitamins by assaying the color changes in different reactions.

Serum vitamin A was measured using the method described by Neeld and Pearson. Trifluoroacetic acid was reacted with the conjugated double bonds of vitamin A to form a faint blue compound. The color change was assayed with a spectrophotometer. Serum ascorbic acid was measured using the method described by Roe and Kuether and modified by Roe. Ascorbic acid is converted to dehydroascorbic acid by shaking with Norit and this was coupled with 2,4-dinitrophenylhydrazine in the presence of fluorourea as a reducing agent. The dinitrophenylhydrazine thus formed is converted by sulfuric acid into a red compound, which can be assayed by a spectrophotometer.

Serum vitamin E was measured by the Emmerie-Engel reaction based on the method described by Baker and Frank. This method is based on the reduction of ferric acid by tocopherols to form ferrous ions which then forms a complex with α-, α-dipyridyl, which was then assayed by spectrophotometry.[17]

The data obtained were analyzed using the Statistical Package for the Social Sciences, version 15.0 (SPSS15). Differences between the two groups were analyzed for statistical significance using the student t-test, odd ratio, and logistic regression, where applicable. Statistical significance was determined at $P<0.05$.

## Results

There was a statistical significant difference in the mean serum levels of vitamins A, C, and E in the oral cancer patients compared with the normal patients ($P=0.022$, $P=0.000$ and $P=0.013$, respectively) [Table 1].

Table 2 shows the association between low serum levels of antioxidant vitamin and oral cancer. Only 6.7% of the normal patients had low serum vitamin A levels (<0.5 μg/l) compared with 43.8% of oral cancer patients who had low serum vitamin A levels. A total of 3.3% of normal patients had low serum vitamin C levels (<0.5 mg/dl) while 25.8% of oral cancer patients had low serum vitamin C levels (<0.5 mg/dl). Most patients in the two groups had low serum levels of vitamin E (<10 mg/l) with 96.6% of the cancer patients and 63.3% of the control group having low serum vitamin E levels.

The risk of oral cancer was 10.89, 11.35, and 5.6 times more in patients who had low serum vitamins A, C, and E, respectively [Table 2]. However, on logistic regression analysis, only low serum vitamin E could independently predict occurrence of oral cancer [Table 3].

Twelve (37.5%) of the cancer patients were males while 20 (62.5) were female whereas, in the control group, 17 (56.7%) were males and 13 (43.3%) were females. In the oral cancer group, the mean serum vitamins levels were consistently higher in females compared with their male counterparts, though differences were not statistically significant ($P=0.73$, $P=0.21$, and $P=0.10$). The mean serum vitamin C level was higher in males than that in females in the control group, but females in this group had higher mean serum vitamins A and E; these differences were also not statistically significant ($P=0.51$, $P=0.63$, $P=0.95$) [Table 4].

**Table 1: Comparison of serum vitamins of oral cancer and control patients**

| Micronutrients | Cancer Mean±SD | Control Mean±SD | *P* value |
|---|---|---|---|
| Serum vitamin A | 0.53 ± 0.26 | 0.81 ± 0.28 | 0.022 |
| Serum vitamin C | 0.63 ± 0.28 | 0.91 ± 0.25 | 0.000 |
| Serum vitamin E | 6.68 ± 3.03 | 8.80 ± 3.48 | 0.013 |

**Table 2: Association between low antioxidant levels and the risk of oral cancer**

| | Oral cancers (*n*=32) | | Control (*n*=30) | | *P* | OR | 95.0% CI for OR |
|---|---|---|---|---|---|---|---|
| | *n* | % | *n* | % | | | |
| Low vitamin A | 14 | 43.8 | 2 | 6.7 | 0.001* | 10.89 | 2.21, 53.69 |
| Low vitamin C | 9 | 28.1 | 1 | 3.3 | 0.013* | 11.35 | 1.34, 96.18 |
| Low vitamin E | 29 | 96.6 | 19 | 63.3 | 0.015* | 5.60 | 1.37, 22.73 |
| Alcohol intake | 8 | 25.8 | 7 | 24.1 | 0.881 | 1.09 | 0.34, 3.53 |
| Tobacco intake | 6 | 23.1 | 2 | 6.9 | 0.131* | 4.05 | 0.74, 22.20 |

*Fisher exact test was used; OR = Odds ratio

**Table 3: Logistic regression analysis of risk factors for oral cancer**

| | B | *P* | OR | 95.0% CI for OR |
|---|---|---|---|---|
| Low vitamin E | −2.672 | 0.041 | −2.67 | −5.30, −0.10 |
| Low vitamin A | −1.791 | 0.063 | −1.79 | −3.69, 0.10 |
| Low vitamin C | −0.907 | 0.486 | −0.92 | −3.44, 1.64 |
| Alcohol intake | 0.559 | 0.505 | 0.56 | −1.08, 2.20 |
| Tobacco intake | −2.437 | 0.062 | −2.44 | −4.96, 0.24 |
| Constant | 3.076 | 0.015 | | |

B = Coefficient of regression, OR = Odds ratio, CI = Confidence interval

**Table 4: Mean serum vitamin level according to gender**

| Serum Indices | Sex | (MSL±SD) cancer | $P_1$ | (MSL±SD) control | $P_2$ |
|---|---|---|---|---|---|
| Vitamin A | Male | 0.51 ± 0.20 | 0.73 | 0.78 ± 0.33 | 0.51 |
| | Female | 0.54 ± 0.30 | | 0.85 ± 0.19 | |
| Vitamin C | Male | 0.56 ± 0.18 | 0.21 | 0.93 ± 0.23 | 0.63 |
| | Female | 0.67 ± 0.32 | | 0.89 ± 0.27 | |
| Vitamin E | Male | 5.67 ± 1.92 | 0.10 | 8.76 ± 4.09 | 0.95 |
| | Female | 7.32 ± 3.45 | | 8.85 ± 2.51 | |

MSL = Mean serum level, $P_1$ = *P* value for cancer, $P_2$ = *P* value for control

Only 26.1% of the oral cancer patients in this study use tobacco, while 25.8% consume alcohol and 12.5% use both alcohol and tobacco. There was, however, no statistically significant difference in tobacco use in the oral cancer group and the controls ($P=0.106$).

## Discussion

In this study, mean serum levels of vitamins A, C, and E were significantly lower in oral carcinoma cases compared to the control. Most studies are in agreement with this finding.

Abiaka *et al.*[18] found the α-tocopherol concentration to be significantly lower in stomach, colon, rectal, and breast cancer cases compared to controls. Choi *et al.*[19] found that the serum level of ascorbic acid in gastric carcinoma patients in Seoul was less than one-fifth of their control. They also found that serum levels of beta-carotene and alpha-tocopherol of gastric cancer patients were significantly decreased compared to those in their control group.

Zheng *et al.*[13] in Maryland, US, observed that serum level of beta-carotene was lower in subjects that subsequently developed oral and pharyngeal cancer. This was also corroborated by Kune *et al.*[13] who observed in a study in Australia that serum vitamin A was significantly lower in oral and pharyngeal cancer cases compared to controls. However, Nagao *et al.*,[14] in a study in Japan, found serum β-carotene to be significantly lower in oral leukoplakia than controls in males, but in females, no significant difference was noted in the serum α-tocopherol and β-carotene levels in leukoplakia patients compared to controls.

Research has demonstrated the roles of vitamins in cellular changes. Vitamin A has particularly been found to influence cellular changes in a number of ways. It inhibits terminal differentiation of epidermal cells; it enhances cellular immunity by promoting an increase in the number of T-helper cells and NK cells. Vitamin A helps in the arrest and reversal of leukoplakia progression. In addition, vitamin A also induces cytotoxic and cytostatic effects on cancer cells and promotes apoptosis as well as interfering with cancer DNA and RNA gene expression.[20] Vitamin C, however, reduces vitamin E degradation and enhances chemotaxis, phagocytosis, and collagen synthesis. It inhibits nitrosamine formation and reduces oncogene expression. Vitamin E is a free radical scavenger; it maintains membrane integrity and inhibits cancer cell growth and differentiation. It also inhibits mutagenicity and nitrosamine formation. Synergism between vitamin E, selenium, and ascorbate inhibits DNA and RNA protein synthesis in cancer cells.[20]

In this study, low serum vitamins A, C, E levels were associated with 10.89, 11.35, and 5.6 times increased risk of oral cancer respectively. This is in agreement with the study of Nagao *et al.*[14] who found high serum micronutrient to be associated with reduced risk of oral leokoplakia in Japanese males. Zheng *et al.*[13] also reported that persons in the highest tertile of total carotenoids had about one-third the cancer risk as those in the lowest tertile, but the risks were elevated significantly with increasing serum levels of alpha-tocopherol.

In this study, only low serum vitamin E independently predicted occurrence of oral cancer. Previous studies have shown that topical and systemic administration of vitamin E inhibited experimental tumors in hamster pouch.[9,10] In addition, Knert *et al.*[21] found that the high

serum alpha-tocopherol level was associated with reduced risk of cancer in Finnish men. However, this differs from the findings of Ramaswamy *et al.*,[22] who found that serum levels of vitamins A and C were significantly lower in oral leukoplakia cases compared to controls but no significant difference was observed in the serum levels of vitamin E in oral leukoplakia cases compared to controls.

The low serum levels of vitamins A, C, and E in patients with oral epithelial cancers may be due to low or improper consumption of vitamin containing foods thereby reducing the protective effects of antioxidants against cancer. The low serum levels may also be due to loss of appetite that may be caused by tumor necrosis factor (TNF) and interleukin 6 (IL-6) produced in cancer patients consequently leading to general malnutrition, including reduced intake of vitamins.[23]

This study showed that the serum levels of vitamins A, C, and E in oral cancer patients were significantly lower than those of healthy volunteers and the risk of oral cancer was higher in patients with low serum antioxidant vitamins.

It is believed that further studies using a larger sample size and cohort studies with long-term follow-up of subjects is desirable. In addition, genetic studies of oral cancer patients may be necessary in this environment where many oral cancer patients give a negative history of exposure to tobacco and alcohol.

## References


1. Sankaranarayanan R. Oral cancer in India: An epidemiologic and clinical review. Oral Surg Oral Med Oral Pathol 1990;69:325-30.
2. Krutchkoff DJ, Chen JK, Eisenberg E, Katz RV. Oral cancer: A survey of 556 cases from the University of Connecticut Oral Pathology biopsy service 1975-1986. Oral Surg Oral Med Oral Pathol 1990;70:192-8.
3. Rudolph P, Atvis K. Ulcerative conditions. In: Regezi J, Sciubba J, Jordan R, editors. Oral Pathology Clinical Pathologic Correlations. Missouri: Saunders; 2003. p. 52-5.
4. Macfarlane GJ, Boyle P, Evstifeeva TV, Robertson C, Scully C. Rising trends of oral cancer mortality among males worldwide: The return of an old public health problem. Cancer Causes Control 1994;5:259-65.
5. Zakrzewska JM. Oral Cancer. Br Med J 1999;318:1051-4.
6. Sugerman PB, Savage NW. Current concepts in oral cancer. Aust Dent J 1999;44:147-56.
7. Feldman JG, Hazan M. A case-control investigation of alcohol, tobacco and diet in head and neck cancer. Prev Med 1975;4:444-463.
8. Weisburger JH. Nutritional approach to cancer prevention with emphasis on vitamins, antioxidants, and carotenoids. Am J Clin Nutr 1991;53(1 Suppl):226S-37S.
9. Suda D, Schwartz J, Shklar G. Inhibition of experimental oral carcinogenesis by topical beta-carotene. Carcinogenesis 1986;7:711-5.
10. Odukoya O, Hawach F, Shklar G. Retardation of experimental oral cancer by topical vitamin E. Nutr Cancer 1984;6:98-104.
11. Winn DM, Ziegler RG, Pickle LW, Gridley G, Blot WJ, Hoover RN. Diet in the aetiology of oral and pharyngeal cancers among women from southern United States. Cancer Res 1984;44:1216-22.
12. Block G. Vitamin C and cancer prevention: The epidemiologic evidence. Am J Clin Nutr 1991;53(1 Suppl):270S-82S.
13. Zheng W, Blot WJ, Diamond EL, Norkus EP, Spate V, Morris JS *et al.* Serum micronutrients and the subsequent risk of oral and pharyngeal cancer. Cancer Res 1993;53:795-8.
14. Nagao T, Ikeda N, Warnakulasuriya S, Fukano H, Yuasa H, Yano M, *et al.* Serum antioxidant micronutrients and risk of oral leukoplakia among Japanese. Oncol 2000;36;466-70.
15. Oji C, Chukwuneke F. Oral cancer in Enugu, Nigeria, 1998-2003. Br J Oral Maxillofac Surg 2007;45:298-301.
16. Lawoyin JO, Aderinokun GA, Kolude B, Adekoya SM, Ogundipe BF. Oral cancer awareness and prevalence of risk behaviours among dental patients in South-western Nigeria. Afr J Med Med Sci 2003;32:203-7.
17. Varley H. Practical clinical biochemistry. London: William Heinemann; 1976. p. 216-22.
18. Abiaka C, Al-Awadi F, Al-Sayer H, Gulshan S, Behbehani A, Farghally M, *et al.* Serum antioxidant and cholesterol levels in patients with different types of cancer. J Clin Lab Anal 2001;15:324-30.
19. Choi MA, Kim BS, Yu R. Serum antioxidant vitamins levels and lipid peroxidation in gastric carcinoma patients. Cancer Lett 1999;136:89-93.
20. Enwonwu C O, Meeks V I. Bionutrition and oral cancer in humans. Crit Rev Oral Biol Med 1995;6:5-17.
21. Knekt P, Aromaa A, Maatela J, Aaran RK, Nikkari T, Hakama M, *et al.* Serum vitamin E and risk of cancer among Finnish men during 10-year follow- up. Am J Epidemiol 1988;127;28-41.
22. Ramaswamy G, Rao V R, Kumaraswany S V, Anantha N. Serum vitamins status in oral leukoplakias: A preliminary study. Eur J Cancer B Oral Oncol 1996;32:120-2.
23. Richter E, Connelly RR, Moul JW. The role of pretreatment serum albumin to predict pathologic stage and recurrence among radical prostatectomy cases. Prostate Cancer Prostatic Dis 2000;3:186-90.


**How to cite this article:** Lawal AO, Kolude B, Adeyemi BF, Lawoyin JO, Akang EE. Serum antioxidant vitamins and the risk of oral cancer in patients seen at a tertiary institution in Nigeria. Niger J Clin Pract 2012;15:30-3.

**Source of Support:** Nil, **Conflict of Interest:** None declared.